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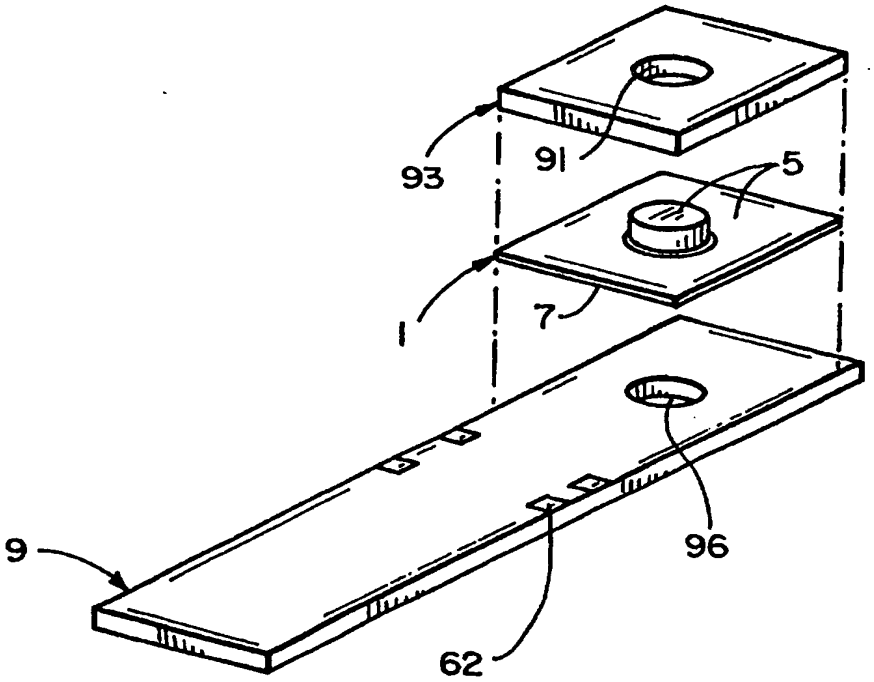
(54) Title: METHODS AND DEVICES FOR DETERMINATION OF AN ANALYTE IN BODY FLUID

(57) Abstract

Devices and methods for utilizing dry chemistry dye indicator systems for body fluid analysis such as glucose level in whole blood are provided by incorporating a porous membrane with a skin side which enables separation of whole blood and visually reading the indicator without removing the red blood cell portion of the blood from the membrane. The devices also enable visual reading of the indicator by use of a membrane or matrix which provides separation of whole blood in a lateral flow of the blood through the matrix from the input area to a test area of the matrix. The devices also provide for microtitration of fluid samples in fixed volumetric openings containing indicator reagent. Another aspect of the device provides a determinati n of hematocrit level in whole blood in combination with indicator indication of analyte concentration which can be compensated for the hematocrit level. The devices provided are low cost due to efficient manufacturing methods provided.



METHODS AND DEVICES FOR DETERMINATION OF AN ANALYTE IN BODY FLUID

FIELD OF THE INVENTION

The present invention relates to a test device and method for the colorimetric determination of a chemical or biochemical component (analyte) in an aqueous body fluid, such as whole blood. In particular the present invention relates to a dry reagent test strip from which an analyte presence and/or concentration is determined by visual interpretation or through the use of an instrument. A common use of such test strips is for determination of glucose level in blood by diabetics.

BACKGROUND OF THE INVENTION

Numerous devices have been developed to test for presence and quantity of analytes in aqueous samples, such as whole blood or urine. The patent and technical literature of the last thirty years is replete with inventions which utilize a reagent strip containing a dry chemistry reagent system, that is, a system in which the wet chemistries are imbibed into an absorbent or bibulous medium, dried, and later reconstituted by fluid from the test sample. The reagent strips contain an indicator which changes color, depending on the presence or concentration of a particular analyte in a biological fluid applied to the strip. These strips may be read visually by reference to a color standard or colorimetrically by instrument calibrated or programmed to detect a certain color. Although some of these strips use reduction chemistries, more commonly they involve an oxidizable dye or dye couple. Some of the strips include an enzyme, such as glucose oxidase, which is capable of oxidizing glucose to gluconic acid and hydrogen peroxide. They also contain an oxidizable dye and a substance having peroxidative activity, which is capable of selectively catalyzing oxidation of the oxidizable dye in the presence of hydrogen peroxide. (See, for example, U.S. Pat. No. 5,306,623, to Kiser et al.) Examples of these devices, in addition to those used to test blood glucose, include tests for cholesterol, triglycerides, calcium or albumin in whole blood, and for protein, ketones, albumin or glucose in urine.

U.S. Pat. 3,552,928, issued to Fetter discloses the use of certain water soluble salts and amino acids in reagent formulations as separation agents to provide blood separation. With solids such as red blood cells substantially removed from the biological fluid, there is less background color at the test site to obscure a change in coloration produced by a testing reagent.

Phillips et al., U.S. Pat. No. 4,935,346 discloses a system wherein a whole blood sample is applied to the device and indicator development occurs in the presence of the colored components of the sample. Measurements of the color change in indicator are made at two distinct wavelengths to eliminate the interferences from the presence of colored blood components.

Kiser et al., in U.S. Pat. Nos. 5,306,623 and 5,418,142, disclose a visual meter device which incorporates various coatings on a matrix material to filter red blood cells from fluids. Similar devices for visual indication are disclosed by Hochstrasser in U.S. Patent Nos. 3,964,871 and 4,059,407.

Terminello et al., U.S. Pat. No. 4,774,192, disclose a system in which the matrix is formed of an asymmetric material used to filter the red blood cells in the sample. The asymmetric material has a density gradient from one side to the other to progressively separate red blood cells from the fluids.

Daffern et al., U.S. Pat. No. 4,994,238, disclose a test device that comprises an asymmetric reagent layer that has progressively finer filtration with increased distance from one surface toward the other surface.

Castino et al., U.S. Patent No. 5,456,835 disclose the use of filters formed of ligand modified polymeric film such as polypropylene fibers and polyethersulfone fibers.

Vogel et. al., U.S. Pat. No. 4,477,575, disclose the use of glass fiber material to achieve blood separation through the thickness of the material. Blood is applied to one side of the glass fiber, and relatively clear fluid migrates out of the opposite side. This fluid is delivered to an additional layer where the detection of analytes can occur.

Macho et al., U.S. Patent No. 5,451,350, disclose the use of absorbent channels to distribute sample fluid in multi-zone test devices. Charlton et al.,

side of the matrix is isotropic for uniform distribution therein of fluid received from the skin side and comprises an indicator capable of indicating the presence or concentration of the analyte. The method comprises applying a blood sample to the skin side of the matrix, allowing the fluid to pass through the skin into the isotropic matrix, then reading or measuring on the test side of the matrix the indication provided by the indicator of the presence or concentration of the analyte without removal of the red blood cells from the skin side of the matrix. The skin side is optionally treated with compounds which assist in blocking the passage of red blood cells and allowing passage of substantially clear fluid. Such compounds, or separating agents, can help facilitate the wicking of the clear fluid into the test side of the matrix. However, it is preferred that the skin side of the matrix is inherently hydrophilic which facilitates the passage of fluid through the skin to the test side of the matrix while blocking passage of the red blood cells. This separation of the blood on the skin side and reading or measuring the resultant indication on the test side of the matrix makes the determination of the presence and/or concentration of analyte simpler due to the relative absence of red blood cells at the test site of system and due to the absence of the necessity of removing the red blood cells before taking the desired reading or measurement.

In another aspect this invention provides a device for testing blood for the presence or concentration of an analyte comprising a holder comprising an opening for receiving a blood sample; and a porous matrix comprising a skin side and a test side wherein the skin is capable of blocking the passage of red blood cells and of allowing the passage of blood fluids containing an analyte to the test side of the matrix and wherein the test side of the matrix is isotropic for uniform distribution of fluid received from the skin side. The test side of the matrix comprises an indicator for indicating the presence or concentration of an analyte in the fluid. The matrix is attached to the holder so that the skin side is oriented toward the opening in the holder for receiving the blood sample such that when a blood sample is applied in said opening the blood contacts the skin side of the matrix allowing the blood fluids to pass to the test side of the matrix

passage of fluid containing an analyte to the test side of the matrix positioned in the volumetric opening. It is preferred that the skin side of the matrix member is a material which is inherently hydrophilic and facilitates the passage of the fluid through the skin side to the test side of the member. The device can optionally have a support member with a visual opening at least in part aligned with the opening in the first member whereby the fluid sample can be applied to one opening, the skin can block passage of solids but allow passage of fluid to the test side of the matrix and the analyte can be detected in the test side of the matrix through the other opening. Sequentially or simultaneously the predetermined volumetric size of the opening in the first member provides for a quantitative measurement of the concentration of the analyte in the fluid by enabling titration of a known amount of indicator reagent and a given volumetric quantity of fluid containing the analyte and the color indicator provides a qualitative indication. This invention further comprises methods of using these devices to quantitively measure an analyte in a fluid.

In another aspect this invention provides a method of making a device for testing concentration of an analyte in a fluid comprising providing a first member being substantially noncompressible and having an opening therein of a predetermined volumetric size and providing a porous matrix member which is fluid permeable and is compressible compared to the first member. The method comprises pressing the matrix member against the first member so that a portion of the matrix member protrudes within said opening and a portion of the matrix member is compressed against the surface of the first member adjacent to said opening. Optionally, a support member with an opening aligned with the opening in the first member can be laminated to the first member to position the compressed portion of the matrix between the first member and the support member. Also, optionally the compressed portion of the matrix member can be removed leaving the portion of the matrix member within the opening. The matrix member used in this method of making such devices optionally can have a skin side wherein such a matrix member is positioned in the devices as

In the above embodiments utilizing lateral flow of the fluid, an anisotropic or asymmetric porous matrix can be used. For example, in such a matrix separation of solid components can occur based on decreasing or changing pore size in the matrix. However, in such embodiments an isotropic porous matrix may be employed where uniform sized pores block the passage of solids. In either case, the solids such as red blood cells, introduced at the initial area of the matrix can be held back from the test area of the matrix. If the solids are not adequately blocked and are allowed close to the test area, the solids may cast a shadow or cause color difference in the test area of the matrix. In such cases compensation may need to be made in the reading of the indicator.

In another aspect this invention provides a device for testing for the presence or concentration of an analyte in a fluid sample comprising a member having a first opening for receiving a fluid sample and a second opening for receiving fluid from the first opening wherein the first opening and the second opening are connected by a restricted flow passageway or delivery channel communicating with the first opening and second opening thus enabling the fluid sample to flow from the first opening to the second opening through the restricted flow passageway. This device further comprises a detector for detecting and measuring the rate of initial flow of the fluid from the first opening through the restricted flow passageway towards the second opening. This aspect of the invention also provides a method of using such device wherein the rate of initial flow of fluid through the restricted passageway is measured and correlated to the concentration of a particular concentration of solids (e.g., hematocrit level) in the fluid sample. It has been found that the rate of initial flow of fluid through the restricted flow passageway can be directly correlated to the concentration of an analyte and the fluid. Optionally, in this aspect of the invention the second opening may contain a porous matrix positioned in the second opening comprising an indicator for indicating the presence or concentration of an analyte in the fluid sample entering the matrix. Also, optionally, the porous matrix positioned in the second opening may

Figure 4 is a perspective exploded view of a matrix member positioned between a first member and a support member, where the device comprises a plurality of test sites for one analyte or multiple analyte tests.

Figure 5 is a perspective view of the device of Figure 4 assembled.

Figure 6 is a perspective exploded view of a device having a plurality of test sites for one or more analytes and a delivery channel for delivering fluid from a central sample introduction point to a plurality of test sites.

Figure 7 is a perspective view of the device of Figure 6 assembled.

Figure 8A is a perspective exploded view of a matrix member partially compressed and partially protruding into an opening in a noncompressible member.

Figure 8B is a perspective exploded view of a matrix member partially compressed between a first member and a support member and partially protruding into an opening in the first member.

Figure 9 is a perspective view of the device of Figure 8B assembled.

Figure 10A is a perspective exploded view of the device of Figure 8B having a plurality of test microtitration sites for one analyte or multiple analyte tests.

Figure 10B is a perspective view of the device of Figure 10A assembled.

Figure 11 is a perspective view of a partially compressed matrix with rounded protrusions for extension into openings.

Figure 12 is a perspective exploded view of a device of Figure 10A having a plurality of microtitration test sites for one or more analytes and a delivery channel for delivering fluid from a central sample introduction point to a plurality of test sites.

Figure 13 is a perspective exploded view of the device of Figure 12 assembled.

Figure 14 is a perspective exploded view of the device of Figure 12 wherein the test sites are arranged to provide gravity-aided flow of the fluid sample to the test sites.

Figure 30 is a perspective exploded view of the device of Figure 27 having a delivery channel for delivering fluid from a central sample introduction point to a plurality of test sites.

Figure 31 is a perspective exploded view having a flow rate determinative delivery channel from a sample introduction opening to an opening containing an optional matrix member.

Figure 32 is a perspective view of the device of Figure 31 assembled and optical detectors for measuring the flow rate of fluid moving through the delivery channel.

Figure 33 is a perspective exploded of the device of Figure 31 wherein the delivery channel contains a matrix member.

Figure 34 is a perspective exploded view of a device similar to Figure 20 wherein the delivery channel is formed on the backside of the member containing the sample introduction opening.

Figure 35 is a perspective view of the device of Figure 34 assembled.

Figure 36 is a bottom view or view of the backside of the member containing the sample introduction opening and showing the delivery channel in the device of Figure 34.

Figure 37 is a perspective view of a test strip of Figure 14 and showing user instruction on the back of the strip at the blood application point.

Figure 38 is a front view of the test strip illustrated in Figure 37 showing user indicia for indicator readings.

Figure 39 is a perspective exploded view of the device of Figure 4 containing an individual or discrete matrix member for each fluid receiving opening and test site.

Figure 40 is a perspective view of the device of Figure 39 assembled.

The invention uses membranes from two categories. The first category includes microporous membranes which separates the blood solids from blood fluids. The most preferred microporous membranes are polyethersulfone polymeric membranes which are formed with a skin side which acts as a red blood cell barrier and a matrix side which has uniform pore size for containing indicator reagents. The second category includes cellulose glass fiber composites or polymer based membrane or matrix products which facilitate lateral wicking of fluid and provide separation of blood solids from blood fluids. Vertical separation occurs perpendicular to the application side, through the depth of the material. Lateral separation occurs within the membrane parallel to the surface of the application side. In either category, this invention provides devices which avoid the necessity for meter reading. Due to the separation of red blood cells, these devices provide reliable visual reading of the indicator by the user. The improved separation and visual reading is in part provided by the devices of this invention where the blood solids and red blood cells are maintained in a floating state on the skin side or in some cases in the lateral matrix, which assists in keeping the color from the solids and cells from contaminating the test areas where visual reading of the indicator is desired.

The first membrane type can be treated with separation agents and test reagents. In a preferred embodiment, the membrane is inherently hydrophilic, has a smooth skin side and a rough matrix side which is an isotropic porous matrix. The whole blood is applied to the skin side and the combination of skin characteristics, hydrophilic matrix and separation agents hold the red blood cells on the surface of the skin side while clear fluid and analytes flow into the matrix. The key is that the whole blood must be delivered from the skin side to achieve proper separation. This mechanism creates a titration area in the matrix area free of red blood cells and containing a consistent volume of relatively clear fluid. The hematocrit effect normally found in dry chemistry tests is minimized as long as adequate clear fluid is provided (by the highest hematocrit blood specified) to rehydrate the indicator reagents while the red blood cells are blocked by the skin from entering the matrix. A reservoir is preferably provided

The microtitration concept employed in some aspects of this invention can be explained as a method of controlling the sample volume and the reagent amount to give a consistent titration and therefore consistent and reliable results. The first step is to create a test zone which is bounded. The traditional wet chemistry analysis uses a fixed (premeasured) volume of sample and titrates a quantity of test reagent against that sample. In a dry format the quantity of the test reagent has to be impregnated into the matrix in a ratio proportional to the void volume of the matrix. This can be accomplished many different ways. The sample volume (SV) is the void volume of the matrix (VVM) minus the solids volume remaining in the matrix from the test reagent following wet application and drying or test reagent volume (TRV). The ratio SV/TRV must be constant to provide an accurate titration.

To achieve microtitration the material void volume and the reagent application must be controlled. The device of this invention creates a fixed control geometry which does not permit cross talk between test areas and the sample delivery channel. The microporous membrane has a tendency to wick laterally, which the device in this aspect of the invention prevents. The whole blood is delivered so that it enters the test area matrix from the skin side of the microporous material. The sample may be introduced in any orientation to the laterally wicking materials which may alternatively be employed. The glass fiber material becomes quite fragile when fully wetted. Therefore, it is practical to only impregnate reagents in the test zones. This can be accomplished by using a syringe or needle to discretely apply the reagents in the test area. The most effective way to do this is to preassemble the device and coat the reagents while the cellulose and glass fiber is supported by the front panel of the test strip device. The other materials can be impregnated into the matrix either locally or by general application but in a controlled fashion.

In this invention, the preferred method for controlling the test area geometry is to emboss the membrane into the gasket or molded part, deforming a portion of the membrane into openings in the gasket or molded part and leaving the test areas uncompressed and compressing a portion of the

material. A wetting agent may be applied to the bottom of the capillary channel to facilitate blood flow without the presence of an absorbent material in which the sample may run. High molecular weight polymeric oils work well as wetting agents. A preferred material is dimethylsiloxane ethylene oxide, part number PS073 from United Chemical Technologies. The same effect may be achieved through the use of patterned hydrophilic printing inks, BSI Corporation Photolink™ hydrophilic surface treatment or using CYREX injection molded part. Thin film materials, used for the front and back layers of the strip, are laminated to either side of the gasket-capillary structure. The wetting agent can be applied to the channel by either an air brush or nylon brush applicator and then dried under a heat lamp. Both methods work equally well.

Separating agents are impregnated into the matrix before, during or after the impregnation of test reagents. The specific compounds are selected to enhance the ability of the matrix to separate whole blood into red blood cells and clear fluid. As discussed previously, the preferred matrix materials comprise a microporous polyethersulfone from Gelman, Pall Hemadyne or Ahlstrom cellulose and glass media.

The separating agents which can be impregnated into the matrix may be selected from the following: polyvinyl sulfonic acid (PVSA), polyethylene glycol (PEG), polystyrene sulfonic acid (PSSA), hydroxypropyl cellulose (commercially available as Klucel™), polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), polyacrylic acid (PAA), water soluble salts, citrates, formates and sulfates, amino acids, chitosan (amino sugar), citric acid, phytic acid and malic acid. These materials may be enhanced through combining with silica or clay. The chemical components can include equivalent materials which help to separate whole blood into red blood cells and relatively clear fluid.

Many analytes in blood exist within a narrow range. The largest normal range for any component of whole blood is the fraction of red blood cells in the whole blood, or hematocrit. A healthy individual may have hematocrit ratio between 35 and 55. Persons at high altitudes and newborns often have elevated hematocrit levels, e.g., 60 or above. Sick individuals may experience hematocrit

methoxynaphthol; pyrogallol red(PGR); bromopyrogallol red (BPR); acid green 25 (AG); MBTH and 8-anilino-1-naphthalenesulfonate (ANS); or N-(3-sulfopropyl)aniline and MBTH; or other known and conventional dye system for different analytes. U.S. Patent No. 5,306,623 to Kiser et. al. discloses effective concentrations of a number of useful dye systems.

A preferred dye system is based on the sulfonated form of MBTH, 3-Methyl-6-(M sulfonate)-benzothiazolinone-(2)-hydrazone (MBTH-S) where M is sodium, potassium, ammonium or other equivalent ion, but is preferably sodium. Sulfonation of MBTH to form MBTH-S is disclosed in U.S. Patent 4,101, 381 to Klose. MBTH-S formed as a dye couple with DMAB, ANS or N-(3-sulfopropyl)aniline provides an indicator system which provides a stable color end point in a short period of time. This dye system enables visual reading on a reliable basis without the use of meters or complex timing sequences.

Certain indicators such as MBTH-DMAB continue to change color over time, i.e., the reaction does not reach a stable end point within a reasonable time period. When it is desirable that such an indicator dye system is used, it is important to take the desired readings at specific time after wetting the test strip and beginning the reaction. U.S. Patent No. 5,049,487 to Phillips et al., incorporated herein by reference, describes the use of a change in reflectance of the matrix as a signal that the matrix has been wetted by the sample. In the present invention, the meter design can incorporate two contacts which make contact with the reagent impregnated test pad. When the test pad has been wetted by the application of blood or test sample, a circuit is made and the timing is initiated. The meter can then take readings at the appropriate times as required by the algorithm in the meter. Alternately, sensors in the meter can detect an object, such as a finger or a pipette, over the test matrix in the area to which sample is applied. The timing can be initiated at the time of or shortly after object detection. Either of these approaches enables the design of a simplified, lower cost meter for use where the indication of the dye system must be measured on a time-dependent basis.

read in binary fashion as described in U.S. Pat. No. 3,964,871, issued to Hochstrasser, a plurality of test areas must be designed into the test device. To permit the chemistry to be sensitive to threshold levels of analyte an antioxidant is used to inhibit or intercept the reaction in visual test zones which only change color if the analyte is present in greater quantity than the inhibition chemistry in that zone. They participate in a noncompetitive reaction and are consumed first by the hydrogen peroxide. If the antioxidant is fully consumed by the reaction the dye indicator(s) is oxidized and color is developed in the test matrix. Hochstrasser, U.S. Pat. No. 3,964,891, provides the background to the design and implementation of a urine inhibition test strip. Kiser et al, U.S. Pat. No. 5,306,623, expands this for blood testing. Antioxidants which may be utilized include 2,3,4-trihydroxybenzoic acid, propyl gallate, ascorbic acid, isoascorbic acid, 3,4 dihydroxy cinnamic acid, 3,4 dihydroxy benzaldehyde, Gallic acid and 5,6-diaminouracil. The antioxidant which is preferred in this embodiment is ascorbic acid.

The multi-zone test systems can use various indicating reagent technologies:

indicating dyes and an antioxidant system to provide threshold readings, which can be utilized in multizone nonmetered test formats as described above;

indicating dyes which are consumed by the reaction, i.e. a test zone with more dye will turn off at higher concentrations of analyte than a test zone with less dye; and

indicating dyes which are generated in proportion to the concentration of an analyte, which may be used in a color match system or in conjunction with a meter.

A three level sample device can be used in the present invention based on the chemistry systems described below.

channel formed in a lateral wicking porous material. The layers of preferred embodiments of the invention are fastened with adhesive such as 3M grade 415 pressure sensitive acrylic adhesive. The porous inert material has a low free radical content and is widely used in medical devices.

The various aspects of the invention disclosed herein can best be illustrated by reference to the drawings and the description thereof which follows.

Figures 1 and 2 illustrate a device of this invention which utilizes a porous matrix member **1** to achieve separation of whole blood into red blood cells and relatively clear fluid. Matrix **1** has a skin side **5** and a test side **7** and is attached to holder **49** which contains opening **21**. The matrix is preferably an intrinsically hydrophilic material and is optionally impregnated or coated with separating reagents to facilitate and maximize blood separation. A sample of whole blood is applied to the skin side **5** of matrix **1** through opening **21**. The combination of the skin characteristics of the matrix, the hydrophilic nature and the separating agents provide blocking of the red cells on the surface of the skin side **5** while clear fluid containing the analyte flows through the skin into matrix **1** and to test side **7**. Indicator reagents are present in the matrix, as well as enzymes, hematocrit adjusters, buffers, antioxidants and chelators, which are useful in providing a test device which is capable of determining the level of an analyte in whole blood. The various indicator reagents are known in the art conventionally formulated into reagent cocktails in solvents and applied to matrix **1**. The cocktails for each analyte to be detected are formulated into groups which can coexist in the same pH and solvent solutions conditions. Each indicator or other reagent cocktail is applied to the test side **7** of matrix **1** and dried. When the blood sample wets the reagent present in the matrix, the indicator in the test side of the matrix changes color to provide the desired indication of the analyte, e.g., glucose, in the blood.

As shown in Figure 3A, a drop of blood or other fluid **30** from the users finger or from an applicator may be applied to the device of Figure 2 through opening **21** and the color change may be read on test side **7** by a test instrument

appropriate test sites for reading the indicator indications, or may be entirely transparent. Such a member can also be a composite laminate of an opaque layer, such as aluminum foil, with opening therein and a transparent plastic film, with opening therein and a transparent plastic film that is a solid sheet but provides visual access through the openings in the opaque layer.

Figures 6 and 7 show a device essentially the same as Figures 4 and 5 but having a blood delivery system for distributing a blood sample internally in the device. The blood delivery system is comprised of gasket layer **13** containing openings **621**, laminated to a channel layer **23** containing capillary passageway **25** communicating with notches **33** which form reservoirs above openings **621**. Blood is applied to the device through sample receiving opening **29** in cover member **31**. The blood travels through the capillary passageway **25** which flow may be assisted by a wetting agent applied to the bottom thereof. The capillary passageway **25** is vented by cut outs **24** in the channel layer **23**, which communicates with vent **22** in gasket layer **13**. Blood fills the notches **33** forming reservoirs in the channel layer **23** and passes through openings **621** to the skin side **5** of matrix **1**. Channel layer **23** and gasket layer **13** can be coated with a wetting agent to aid in blood flow through the channel or can be an inherently hydrophilic plastic, such as a sulfonated plastic. The blood is separated into relatively clear fluid which is passed through skin side **5** to test side **7** of matrix **1** and red blood cells which are retained on the surface of skin side **5**. The color formed in the indicator in the test side **7** of matrix **1** is viewed through openings **11** in support member **19**. The device of Figures 6 and 7 is made by laminating cover member **31**, channel layer **23**, gasket layer **13**, matrix **1** and support **19** to form a unitary device. Appropriate adhesives may be used between the various layers to provide adhesion of the layers into the formation of the unitary device of Figure 7 and to provide appropriate sealing of the multiple test zones from each other and to provide a confined internal path for the blood sample to flow from opening **29** through the confined path defined the capillary passageway **25**, notches **33**, openings **621** to the skin side **5** of matrix **1** and prevent any flow of fluid from one test zone to another.

Another embodiment of this aspect of the invention is illustrated in Figure 8A, after matrix member **1** is compressed against member **93** to form the protrusion of matrix member **1** into opening **91** the partially compressed matrix **1** can be removed from member **93** and placed on holder **9** as shown in Figure 8A. In this embodiment the protruding noncompressed portion of partially compressed matrix **1** is inserted into opening **96** to provide a simple device on which a blood sample may be applied to opening **96** and skin side **5** of matrix **1** and the indicator read or measured on test side **7** of matrix **1**. It will further be apparent and understood that in making the device of Figure 9 matrix **1** can be compressed between member **93** and holder **9** in an appropriate lamination process with appropriate adhesives. In such a process opening **96** is temporarily blocked with a tool to prevent matrix **1** from protruding into opening **96** during the lamination and compression.

An important aspect of the device shown in Figure 8B and 9 is that opening **91** is provided to have a predetermined volumetric size. This volumetric opening is substantially filled with the protruding portion of matrix **1** containing an indicator reagent. This configuration thereby provides a specific known and predetermined volume in opening **91** which provides a microtitration chamber of a given volume for a given quantity of indicator in the protruding matrix **1** positioned within volumetric opening **91**. Thus, in addition to an ordinary color indication by an indicator, this device can provide a specific, concentration indication on a titration basis for a known volume of fluid filling volumetric opening **91** and a given amount or concentration of indicator or other reagent present in volumetric opening **91**.

As described above a blood sample applied to the device of Figure 9 or the device of Figure 8A is applied to the skin side of matrix **1** present in volumetric opening **91** or volumetric opening **96**, whereby the red blood cells or other solids are blocked from passage by skin side **5** and the blood fluids are passed through skin side **5** to test side **7** of matrix **1**.

Figures 10A and 10B show a device essentially the same as Figures 8B and 9 but having multiple volumetric openings **91** with multiple portions of

at the top edge it can be seen that gravity will assist in the flow of the blood along capillary passageway **25** and through notches **33** and vents **36**. Figure 16 shows support member **19** having a corresponding arrangement of openings **11** to correspond to the layout of the protrusions of the matrix member **1**. Figures 37 and 38 show the same device with user instructions on one side, i.e., where to apply the blood sample, and indicia on the other side for visual indication of the test results, i.e., level of glucose concentration.

Figures 17, 18, 19 and 20 illustrate a variation of the device of Figures 12 and 13. In this configuration matrix **1** is compressed against member **34** as shown in Figure 17, which results, after removal of the compressed matrix **1** from member **34** in a partially compressed matrix **1** having a protrusion of uncompressed portion of matrix **1** as shown in Figure 18. Figure 19 illustrates the remaining uncompressed portion of matrix **1** after most of the compressed portion of matrix **1** has been removed from around the uncompressed portion leaving element **17** which is a uncompressed shape of matrix **1** having a small border around the base thereof of compressed matrix **1**. These elements may then be assembled into appropriate openings such as the volumetric openings **91** illustrated in Figure 10A. As shown in Figure 20 the matrix elements **17** may be assembled so that they fit into openings in member **35** and sealed by adhesive around the border at the base of each matrix element. This type of device can be assembled and used as described above with respect to the devices of Figures 12, 13 and 14.

Figures 21 and 22 illustrate another aspect of the present invention wherein the porous matrix is utilized in a device having an offset configuration. This device provides for the lateral transfer of the fluid sample through the matrix member to provide certain advantages in the reading or measurement of the indicators. As shown in Figure 21 holder **49** contains opening **21**, matrix **40** is positioned between holder **49** and support **19**, and support **19** contains opening **11** which is laterally offset a given distance from opening **21** in holder **49**. In this device matrix **40** has initial area **47** corresponding to opening **21** and test area **45** corresponding to opening **11**. A sample fluid is introduced through

zones. Otherwise the configuration is similar in that openings **21** correspond to initial area **47** and openings **11** correspond to test area **45** of matrix **40**. The function of the device of Figures 25 and 26 is the same as the device Figures 21 and 22 but on a multiple zone basis.

The device of Figure 27 is essentially the same as the device illustrated and described in Figures 23 and 24 except in a multiple test zone configuration. Similarly the device of Figures 28 and 29 correspond to the device of Figures 25 and 26 but further incorporating the internal capillary passageway distribution system for the fluid as described above in connection with Figures 6 and 7. Similarly, Figure 30 illustrates a device of Figure 27 but with internal capillary passageway distribution system for the fluid.

Figures 31 and 32 illustrate another aspect of the devices of the present invention which enable analysis of an analyte in a fluid by measuring the initial flow rate through a restricted flow passageway. In this device member **323** contains first opening **322** and second opening **366** with restricted flow passageway **325** communicating with the first opening and the second opening, whereby the fluid sample introduced into the first opening **322** will flow by capillary action through passageway **325** to opening **366**. The device further comprises cover layer **331** having opening **321** corresponding to opening **322**. The device further comprises transparent support member **319** having opening **311** corresponding to opening **366**, which can optionally have matrix member **1** compressed into or preshaped to fit into opening **366**. In this device support member **319** is transparent so that the flow of fluid from opening **322** through passageway **325** to opening **366** can be observed and can be measured by detector **64**. Detector **64** is adapted to measure the rate at which the initial flow of fluid occurs from opening **322** through passageway **325**. The rate of flow of the fluid can be correlated to known concentrations of an analyte in the fluid so that measuring the rate of initial flow of a know fluid for a known analyte will provide the concentration of the analyte in the fluid being tested. When the fluid reaches opening **366** and flows into matrix **1** containing appropriate indicator reagents, the typical reaction will occur and the indication of the

is preferred. On the skin side **5** of matrix **1**, the thickness of the skin capable of blocking the passage of red blood cells will be about 0.5 mil or less. The holder member such as **49** in Figures 1 and 2 will generally be a polymeric strip having a thickness from about 5 mils to about 12 mils in most applications and depending on the type of polymeric strip employed a thickness of about 7 to 8 mils is preferred for the holder member. The support member such as **19** in Figure 4 can also have a thickness of from about 5 mils to about 12 mils with about 7 to 8 mils in thickness being preferred when the support member is polymeric. The support member may also be made of a metal foil such as aluminum foil in which case the support member may have a thickness of about 1 to 3 mils in thickness. It will be apparent that when the support member is a metal foil it may be laminated with a transparent plastic film where the openings in the metal foil are appropriately positioned and the transparent film is laminated between the foil and the matrix member where the transparent polymeric film can provide protection of the matrix member containing the indicator reagent from contamination. It will further be recognized that a support member can also be a transparent polymeric strip where the openings are merely visually transparent areas which allow reading or measurement of the indication of the indicator on the matrix member.

Certain member of the devices of this invention such as **93**, **13** and **35** which provide fixed volumetric openings into which the matrix material is compressed will generally be in the range of 4 to 12 mils in thickness and preferably about 4 to 5 mils in thickness. It will also be recognized that these members providing the volumetric fixed size openings will preferably be injection molder materials but can be sufficiently rigid in noncompressible polymeric strips from which the desired volumetric opening has been punched or dye cut.

It will be recognized by those skilled in the art that the overall thickness of the assembled test strip devices according to this invention may vary according to the desired use. The overall thickness of the assembled devices can range from about 8 to about 40 mils. Due to the strength provided by

The methods of assembling the devices according to the present invention will be apparent to one skilled in the art following the teaching contained herein together with conventional laminating techniques for application of adhesive to the various layers, heat bonding various layers and similar techniques for assembly of the devices disclosed herein.

The devices of this invention are conveniently made into test strips of convenient size and configuration particularly for individual use by visual inspection or for use in instruments or meters which are adapted to measure the color or other indication provided by the test strips. It is also convenient to provide the test strip devices of the present invention in a kit form for use by an individual wherein the kit contains a test strip according to the present invention, an antiseptic applicator, an anesthetic applicator, a sharp article for puncturing the skin of the individual to provide a blood sample, and a bandage for the skin puncture site. When supplied in this kit form, proper and consistent use by the individual will be encouraged and facilitated due to the convenience of the kit.

It is desirable to have a system, or kit, which contains all the necessary supplies for performing a test. This is particularly advantageous for diabetics, many of whom are highly mobile. This invention describes a visual test strip which lends itself well to incorporation in a kit. An individually foil wrapped strip coupled with a commercially available disposable lancing device provides the minimum supplies required to perform a blood glucose test. The kit may optionally include a prepackaged towlette to clean and/or numb the test area and an adhesive bandage to cover the lanced site. The presentation of a complete testing kit is extremely useful for individuals as well as for clinics or visiting nurse groups where complete segregation of all testing supplies from patient to patient is advantageous.

One example of a material useful for lateral transfer of fluid containing analyte and blocking lateral transfer of solids is a composite cellulose and glass fiber matrix, such as that available from Ahlstrom Filtration, Inc., Mt. Holly Springs, Pennsylvania, U.S.A., as part number 1661 or 1662, especially to

30 mg horseradish peroxidase, 100 units/mg, and 3.0 glucose oxidase, 2000 units/ml

Stir until dissolved.

Reagent 3a — Antioxidant solution of 50:50 ethanol and ascorbic acid at a pH of 4.0, in varying amounts.

Example B: Test Reagents

Reagent 1b — 20 ml water

420 mg citric acid (a buffering agent). Adjust th pH of the citric acid solution with NaOH to a value of 4.25.

16.7 mg EDTA

90 mg Gantrez S95 available from GAF

250 mg Crotein SPA

20,500 units glucose oxidase

16,200 units peroxidase

Reagent 2b — 10 ml of a mixture of 3 parts by volume water and 7 parts by volume isopropyl alcohol

13 mg MBTH-S

40 mg ANS

Reagent 3b — Antioxidant solution of ethanol and ascorbic acid in varying amounts.

Test A

Polyethersulfone matrix

A piece of polyethersulfone membrane is uniformly coated with reagent 1a; the excess is squeegied off and the material is dried. The membrane is then coated with reagent 2a in the same fashion and dried. The antioxidant solution reagent 3a is directly applied to the test areas in varying concentrations using a syringe. The membrane is then assembled into a test device as shown in Figure 2. Whole blood is applied to the sample opening and the glucose level is read from the front based on the indicator response in each of the test zones.

Pall Hemadyne Membrane

A piece of Pall Hemadyne membrane is uniformly coated with reagent 1b, excess fluid is squeegied off and the material is dried. It is then uniformly coated with reagent 2b in similar fashion and dried. The antioxidant solution reagent 3b is applied discretely to each test area in varying concentrations using a syringe. Whole blood is applied to the sample hole and the glucose level is read from the front.

The dry chemistry reagent system can be used with the identified membranes in many different ways. The system can be used to develop a visual strip for multiple analytes or for varying concentrations of the same analyte. The system can be used for meter read or color match tests. Additional enhancements can be developed by interfacing the strips with a meter and providing novel interface systems for the test device and meter. The following systems could be incorporated into a test device to provide calibration information and start of test signals:

- o Barcode on strip
- o Magnetic strip
- o Notches or magnetic printed areas in the handle of the strip which interface with contacts or reed switches in the meter to provide a binary value, i.e. a 1 equals present and a 0 equals not present. Thus, 16 different settings can be coded into the strip as follows.

We claim

1. A method of testing blood for the presence or concentration of an analyte comprising:

providing a matrix member comprising a skin side and a test side wherein the skin side comprises a porous skin which is a water insoluble polymeric layer capable of blocking the passage of red blood cells and of allowing passage of blood fluids containing an analyte to the test side of the matrix and wherein the test side of the matrix is the opposite side from the skin side and is isotropic for uniform distribution therein of fluid received from the skin side and comprises an indicator capable of indicating the presence or concentration of the analyte;

applying a blood sample to the skin side of the matrix; and

reading or measuring on the test side of the matrix the indication provided by the indicator of the presence or concentration of the analyte without removal of the red blood cells from the skin side of the matrix.

2. A method according to claim 1 wherein the reading or measurement is provided by an instrument.

3. A method according to claim 1 wherein the reading or measurement is provided visually.

4. A device for testing blood for the presence or concentration of an analyte comprising:

a holder comprising an opening for receiving a blood sample; and

a matrix member comprising a skin side and a test side wherein the skin is a water insoluble polymeric layer capable of blocking the passage of red blood cells and of allowing passage of blood fluids containing an analyte to the test side of the matrix and wherein the test side of the matrix is the opposite side from the skin side an is isotropic for uniform distribution therein of fluid received from the skin side;

wherein the matrix member is attached to the holder whereby the skin side is oriented toward the opening in the holder for receiving the blood sample such that when a blood sample is applied in said opening

passage of fluid containing an analyte to the test side of the matrix positioned within said opening to provide indication of the analyte on a volumetric basis.

10. A device according to claim 9 comprising a support member comprising an opening therein on which support member the first member containing the matrix member is mounted such that the opening in the first member is at least in part aligned with the opening in the support member, whereby the device is capable of receiving a fluid through one opening and allowing detection of the indication of the indicator through the other opening.

11. A device according to claim 9 wherein the first member is rigid in comparison to the matrix member and the matrix member positioned in the opening is a discrete shape and size substantially corresponding to the volumetric size of the opening in the first member.

12. A device according to claim 10 wherein the first member is rigid in comparison to the matrix member and the matrix member is compressible and is larger than the opening in the first member whereby a portion of the matrix member is positioned within said opening and a portion of the matrix member is compressed between the first member and the support member.

13. A device according to claim 9 wherein the matrix member comprises a polyethersulfone polymer.

14. A device according to claim 9 further comprising a plurality of openings in the first member, a matrix member positioned within each such opening and a plurality of openings in the support member positioned to correspond to the openings in the first member.

15. A device according to claim 12 further comprising a plurality of openings in the first member, a matrix member positioned within each such opening and plurality openings in the support member positioned to correspond to the openings in the first member and comprising machine readable reference codes on the first member or the support member.

16. A device according to claim 14 wherein the plurality of openings in the first member are interconnected with a capillary passageway and the first

21. A method according to claim 19 wherein the skin side of the matrix is positioned away from the first member.

22. A method according to claim 17 comprising:

providing a skin film capable of blocking passage of solids present in the fluid and allowing passage of fluid containing an analyte; and

laminating the skin film on one surface of the matrix member before or after the matrix member is pressed against the first member.

23. A method according to claim 18 comprising:

providing a skin film capable of blocking passage of solids present in the fluid and allowing passage of fluid containing an analyte; and

laminating the skin film on one surface of the matrix member before or after the matrix member is pressed against the first member.

24. A method according to claim 18 wherein the first member is provided with a plurality of openings, the matrix member is pressed into each opening and the support member is provided with plural openings at least in part aligned with the openings in the first member.

25. A method according to claim 24 wherein the plurality of openings in the first member are interconnected with a capillary passageway and the first member is covered by a cover member having an opening for receiving the fluid sample wherein the opening in the cover member communicates with the capillary passageway thus enabling the fluid to flow from the opening in the cover through the capillary passageway into the interconnected openings in the first member.

26. A method of testing a fluid for the concentration of an analyte present in the fluid comprising:

providing a device according to claim 9;

applying a fluid to the device and allowing the fluid to permeate the matrix member within the opening in the first member and to fill the predetermined volume of the opening in the first member; and

reading or measuring the indication provided by the indicator to provide indication of the analyte on a volumetric basis.

30. A device according to claim 29 further comprising a support member comprising an opening therein on which support member the first member and the matrix member are mounted such that the matrix member is positioned between the first member and the support member and such that the opening in the support member is offset from the opening in the first member and is positioned over at least a portion of the test area of the matrix whereby the device is capable of receiving a fluid through an opening at the initial area of the matrix, allowing the fluid to pass through the matrix from the initial area of the matrix to the test area of the matrix, the pores in the matrix blocking the passage of solids from the initial area of the matrix to the test area of the matrix, and allowing detection of the indication of the indicator through an opening in the support member at the test area of the matrix.

31. A device according to claim 29 wherein the first member comprises a second opening positioned laterally from the initial area of the matrix to correspond to the test area of the matrix member whereby the indication of the indicator can be detected through the second opening.

32. A device according to claim 31 further comprising a support member on which support member the first member and the matrix member are mounted such that the matrix member is positioned between the first member and the support member whereby the device is capable of receiving a fluid through one opening at the initial area of the matrix, allowing the fluid to pass through the matrix from the initial area of the matrix to the test area of the matrix, blocking the passage of solids from the initial area of the matrix to the test area of the matrix, and allowing detection of the indication of the indicator through the second opening in the first member.

33. A method of testing a fluid for the concentration of an analyte present in the fluid comprising:

providing a device according to claim 29;

applying a fluid to the opening for receiving a fluid sample and allowing the fluid to flow through the porous matrix member to the test area;

reading or measuring the indication provided by the indicator.

a bandage for the skin puncture site.

39. A method of initiating the reading and timing sequence of a meter reading a test strip containing an indicator dye system comprising sensing the input of the liquid sample into the test strip positioned in the meter.

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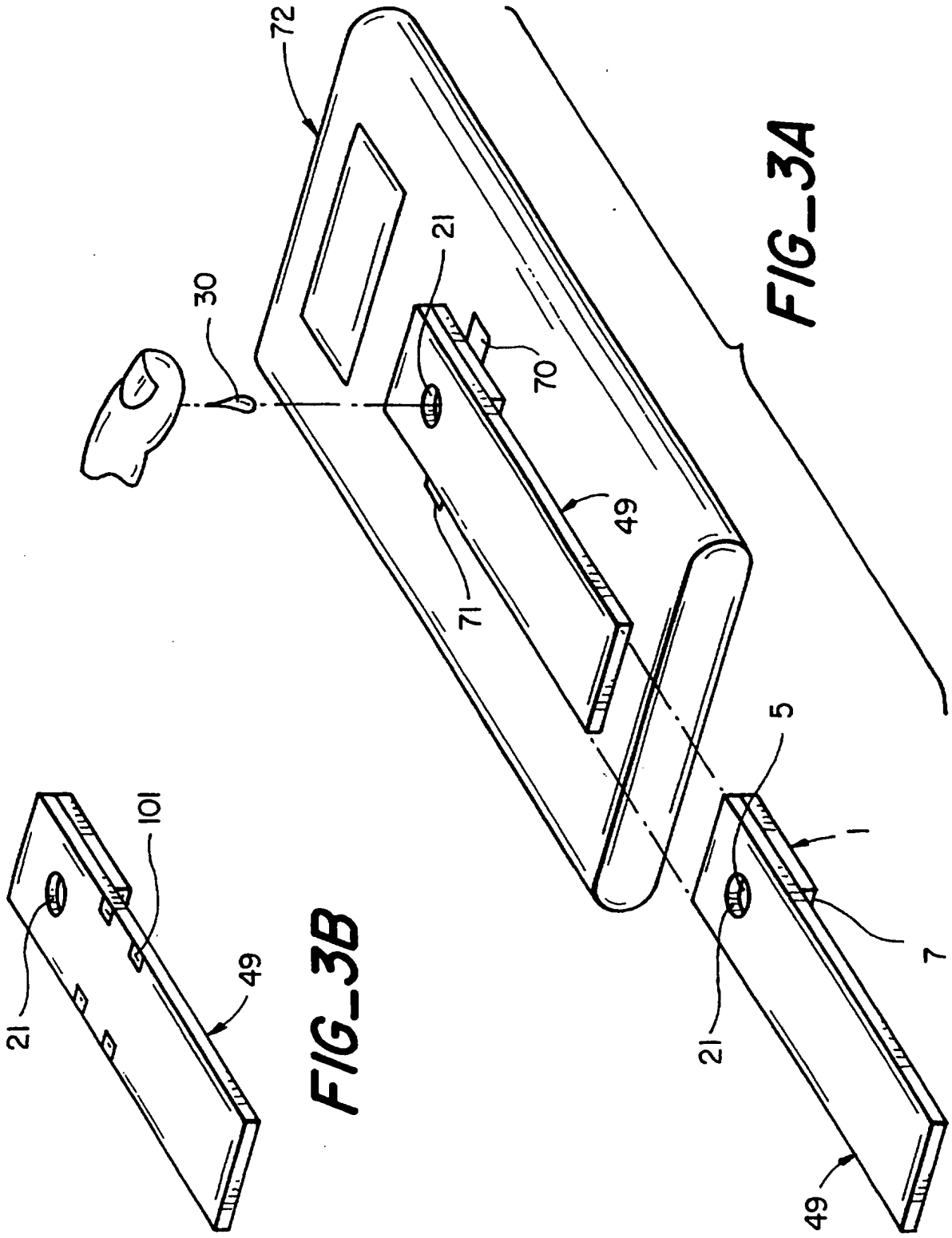


FIG_3A

FIG_3B

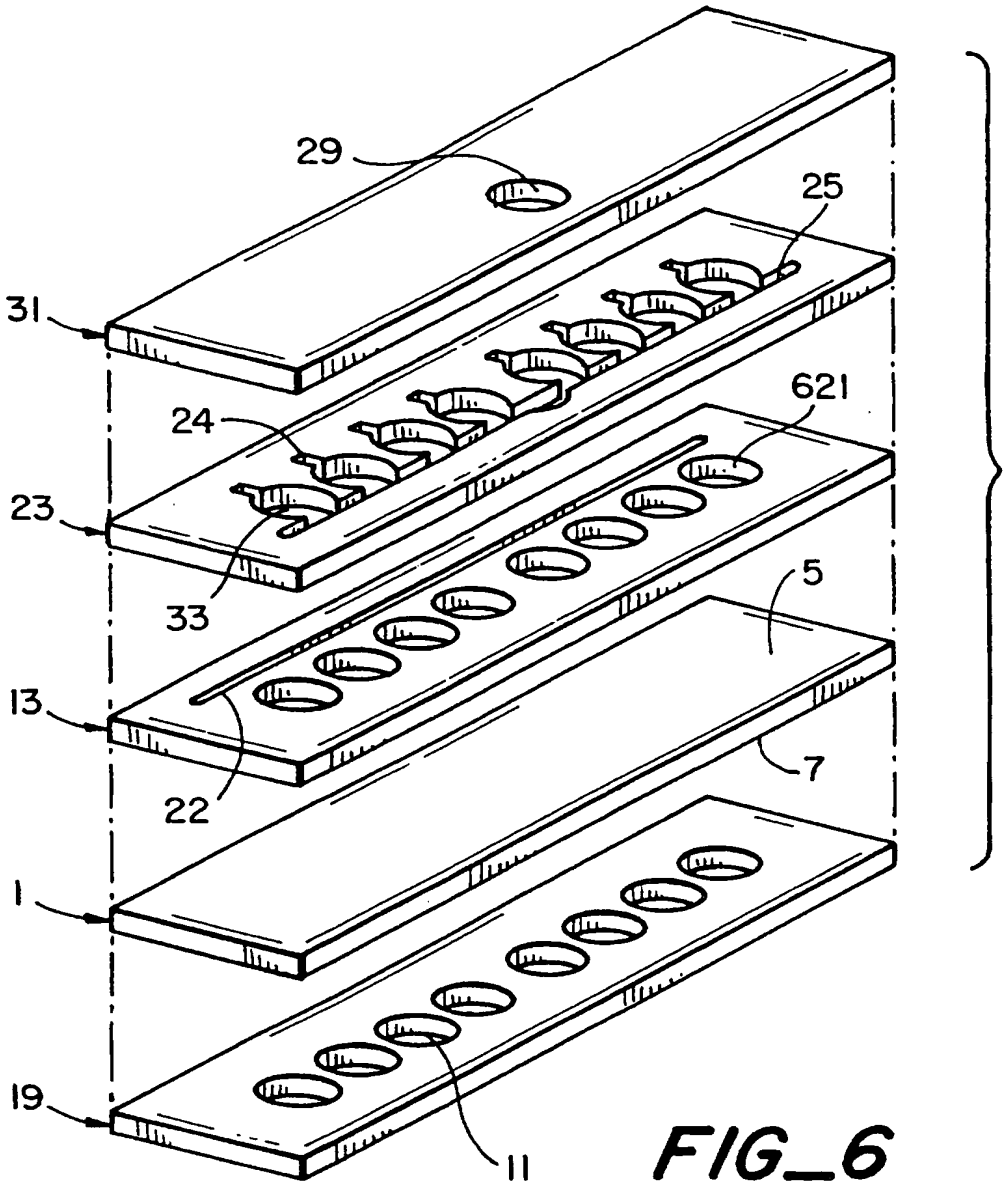


FIG_6

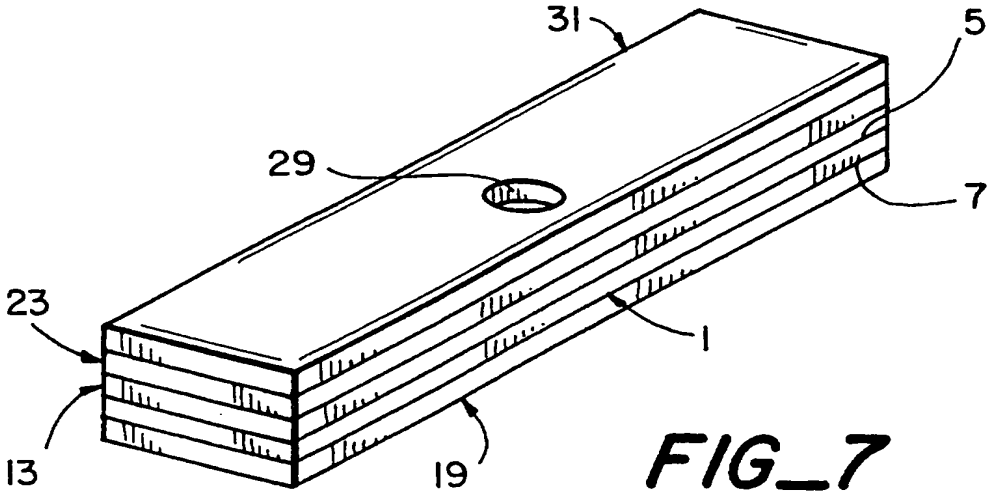


FIG_7

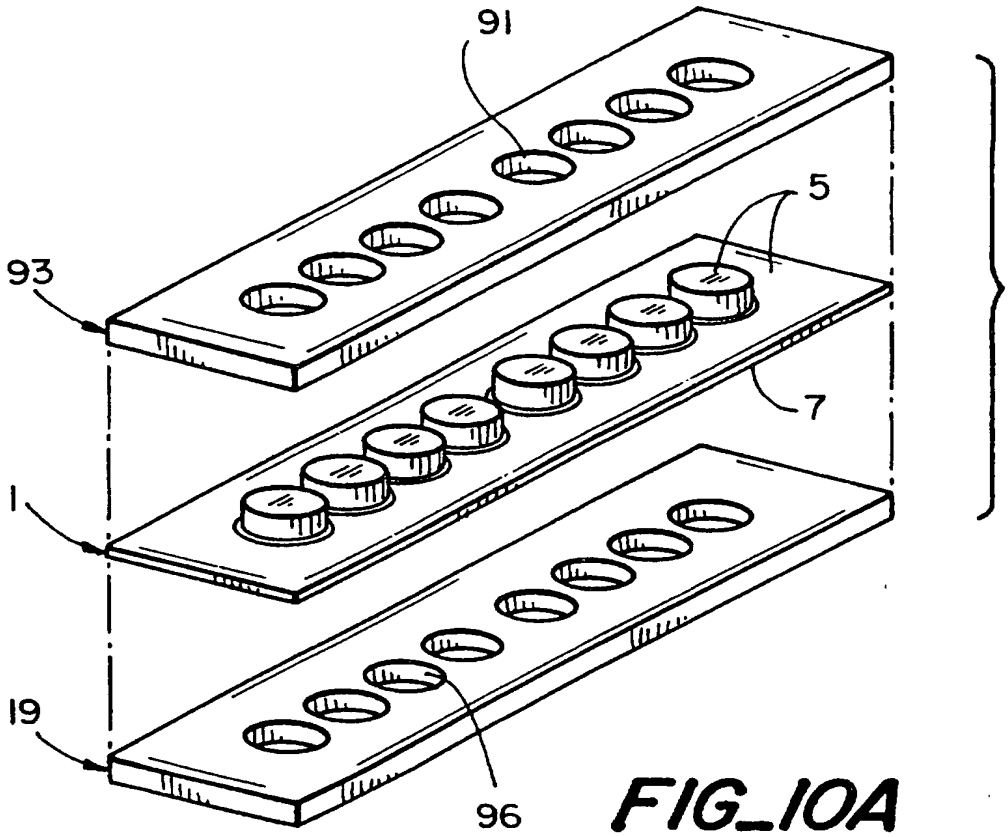


FIG_10A

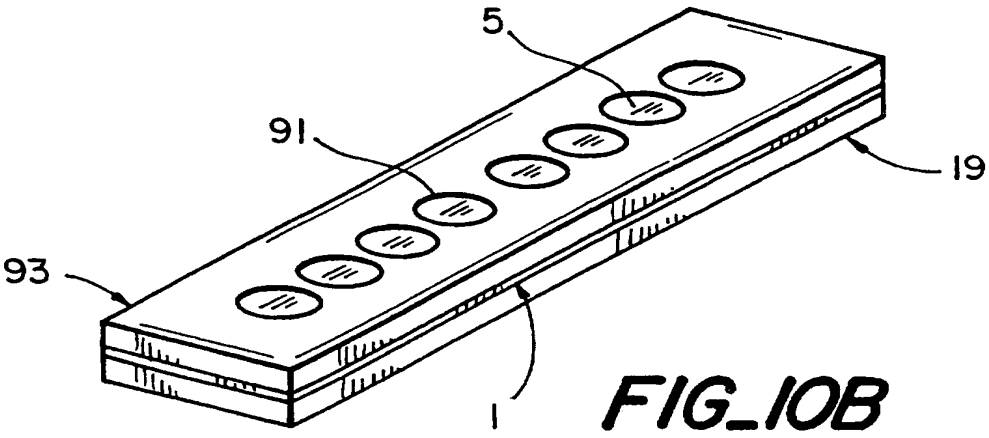


FIG_10B

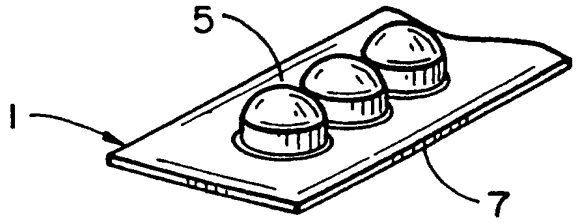


FIG_11

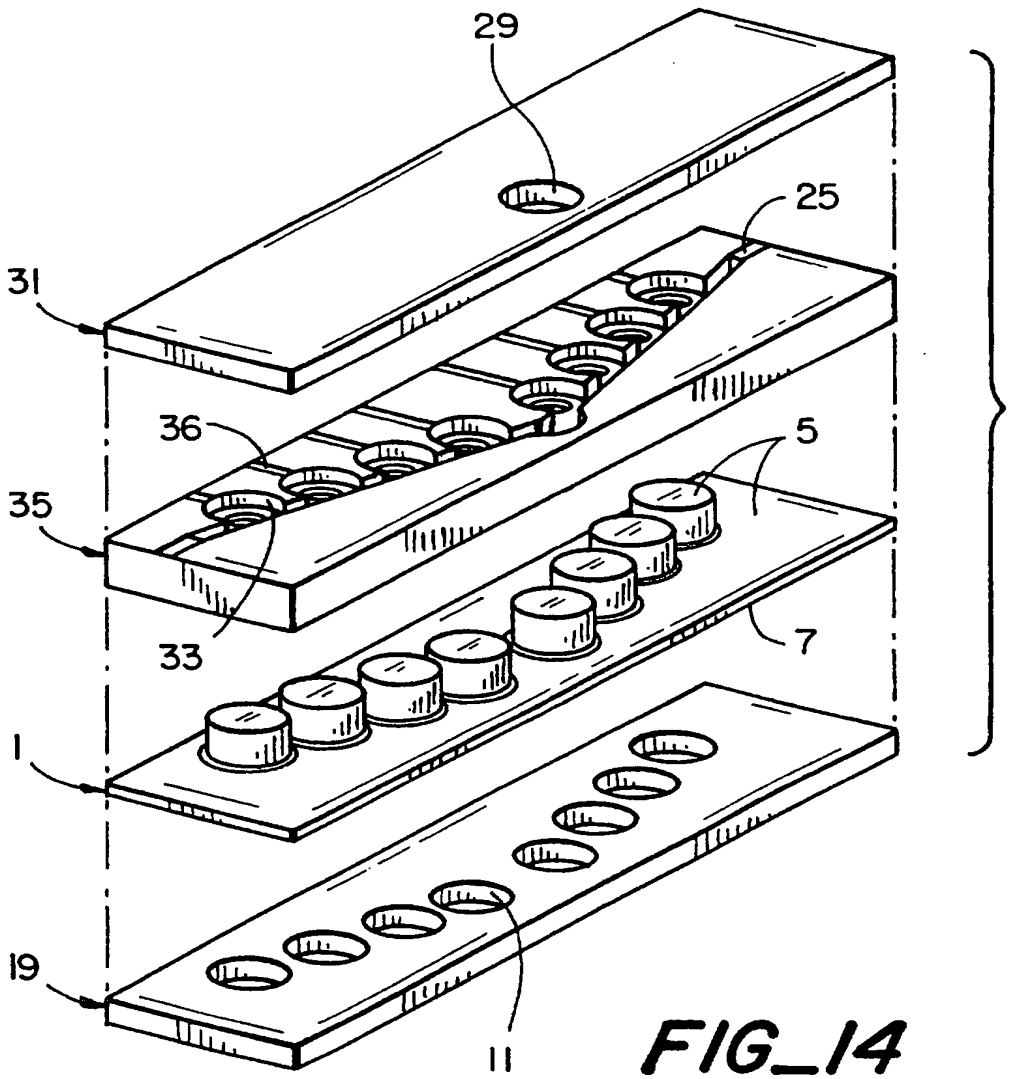


FIG_14

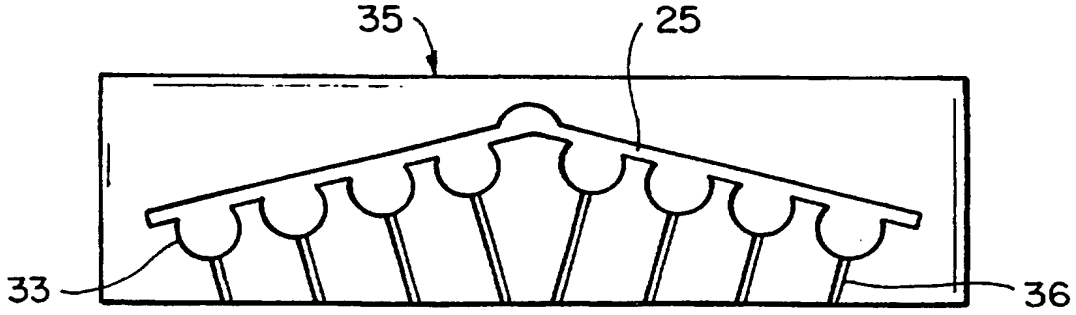


FIG_15

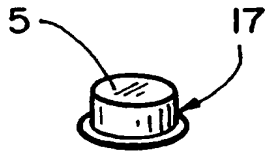


FIG_19

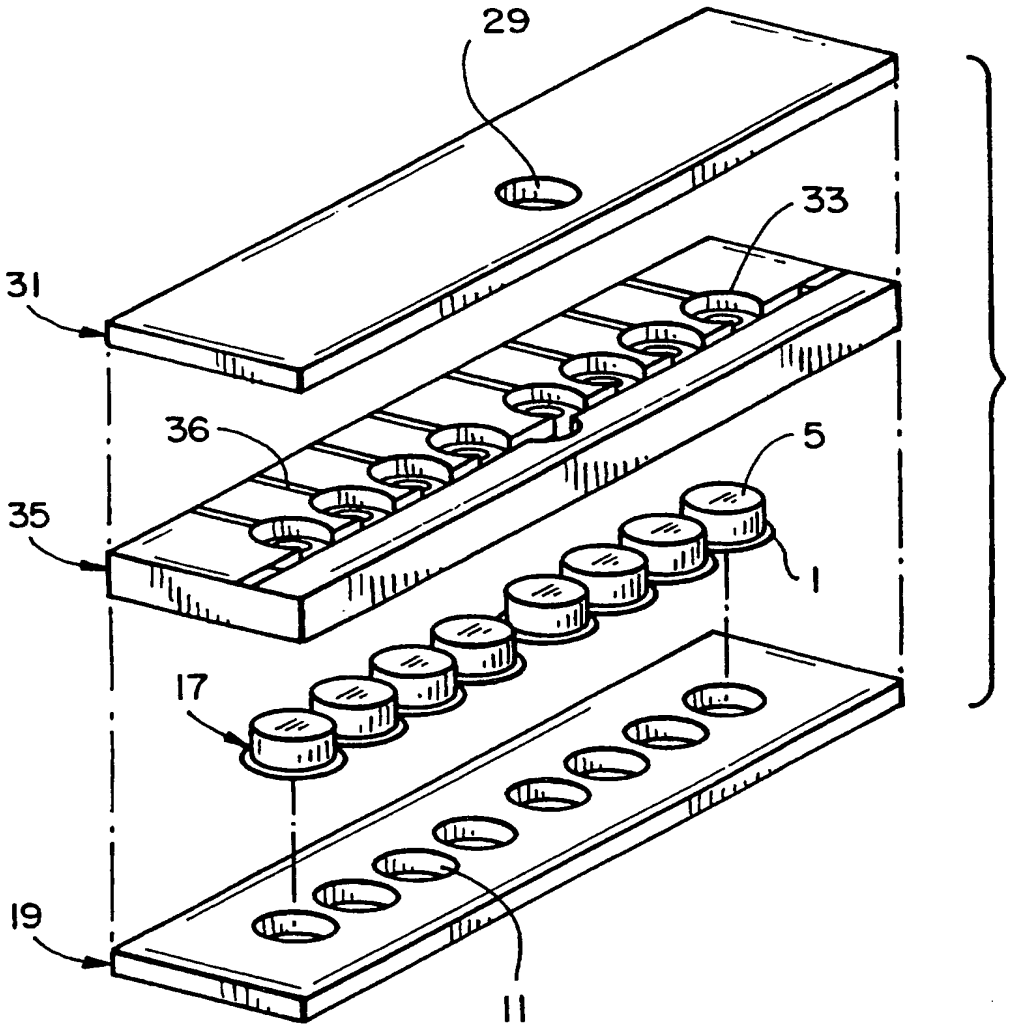
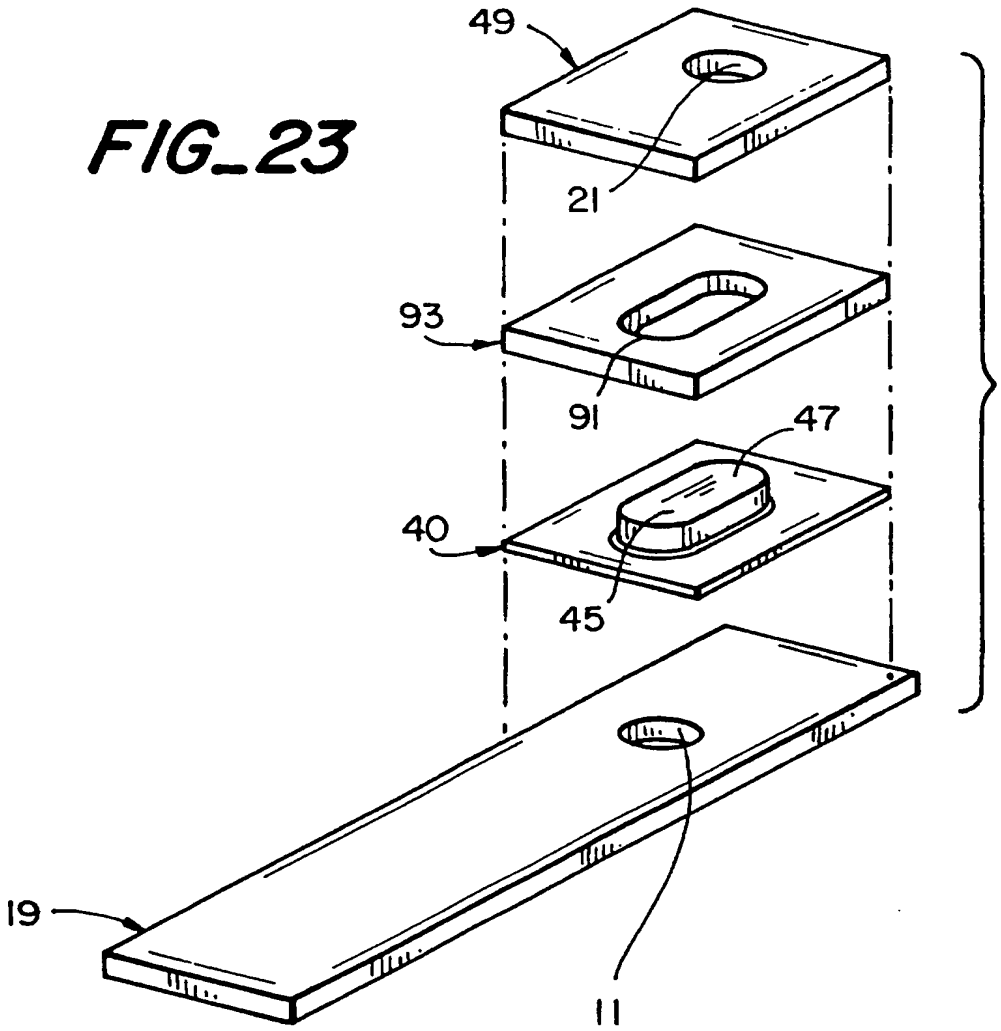


FIG_20

FIG_23



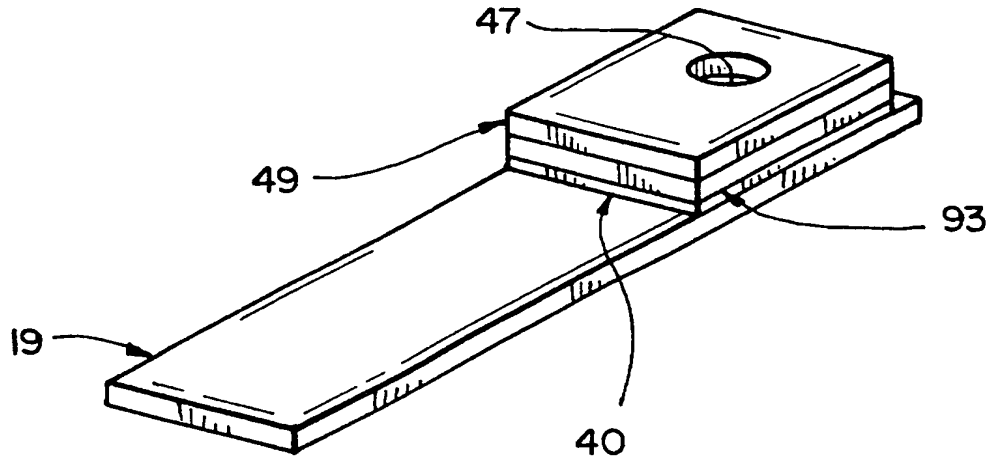


FIG_24

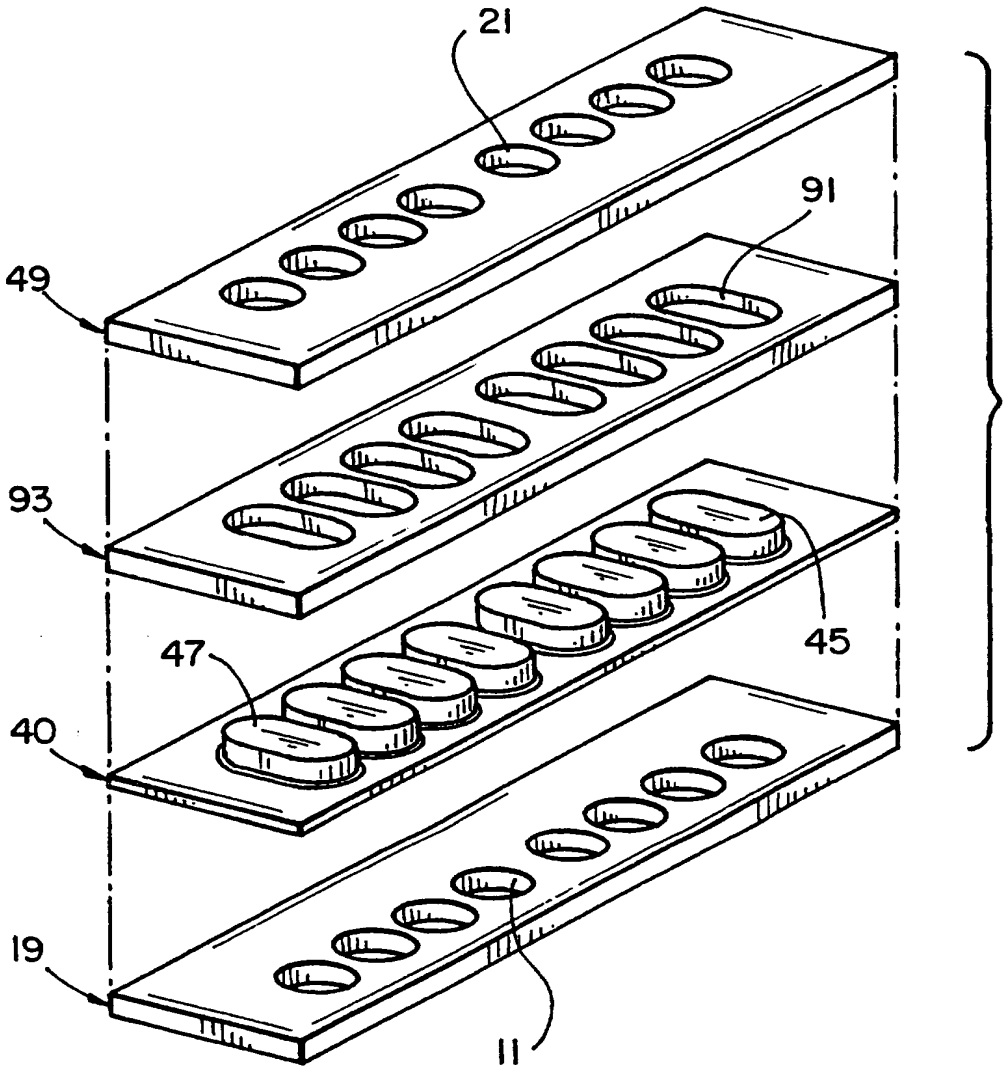


FIG_27

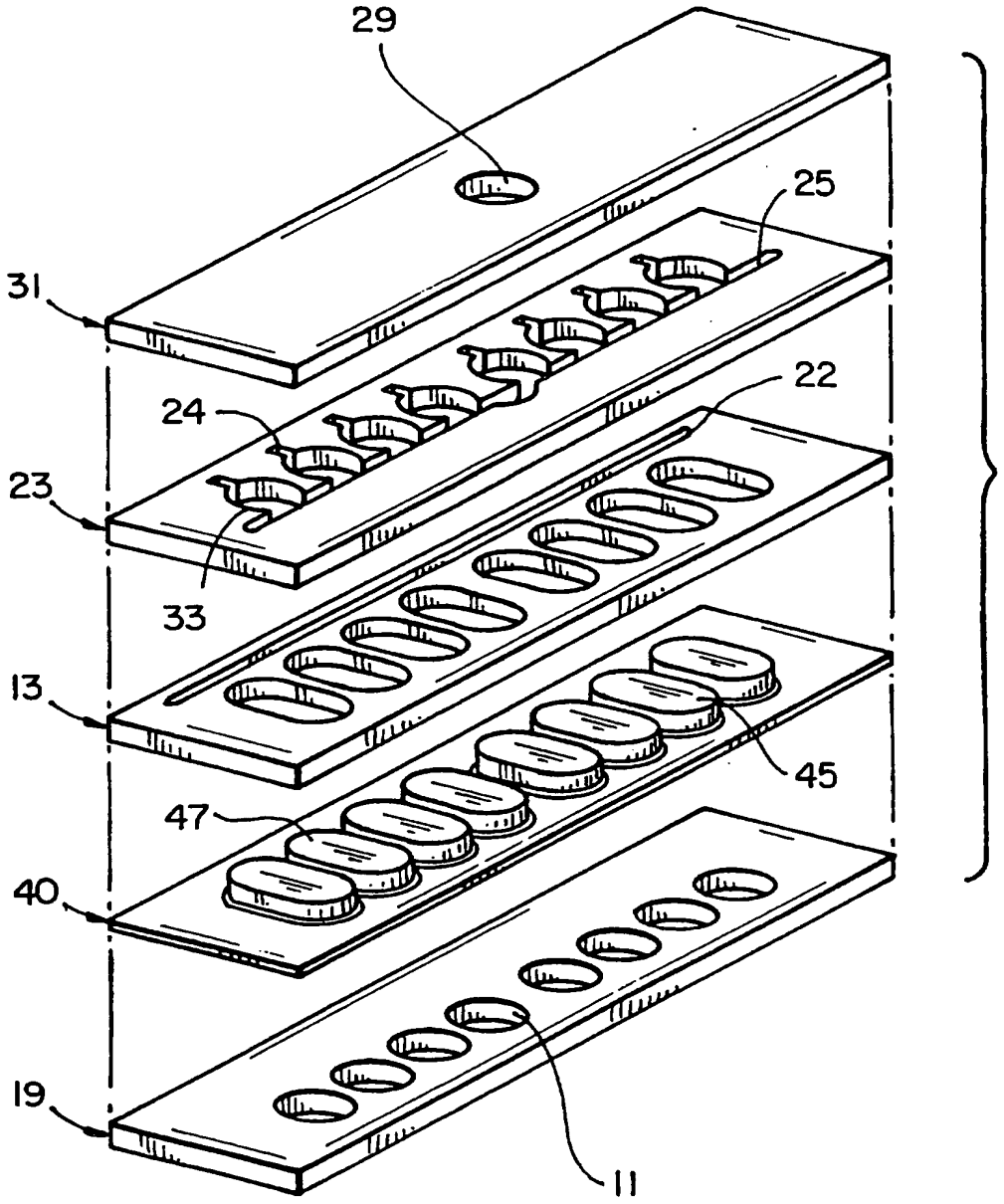


FIG_30

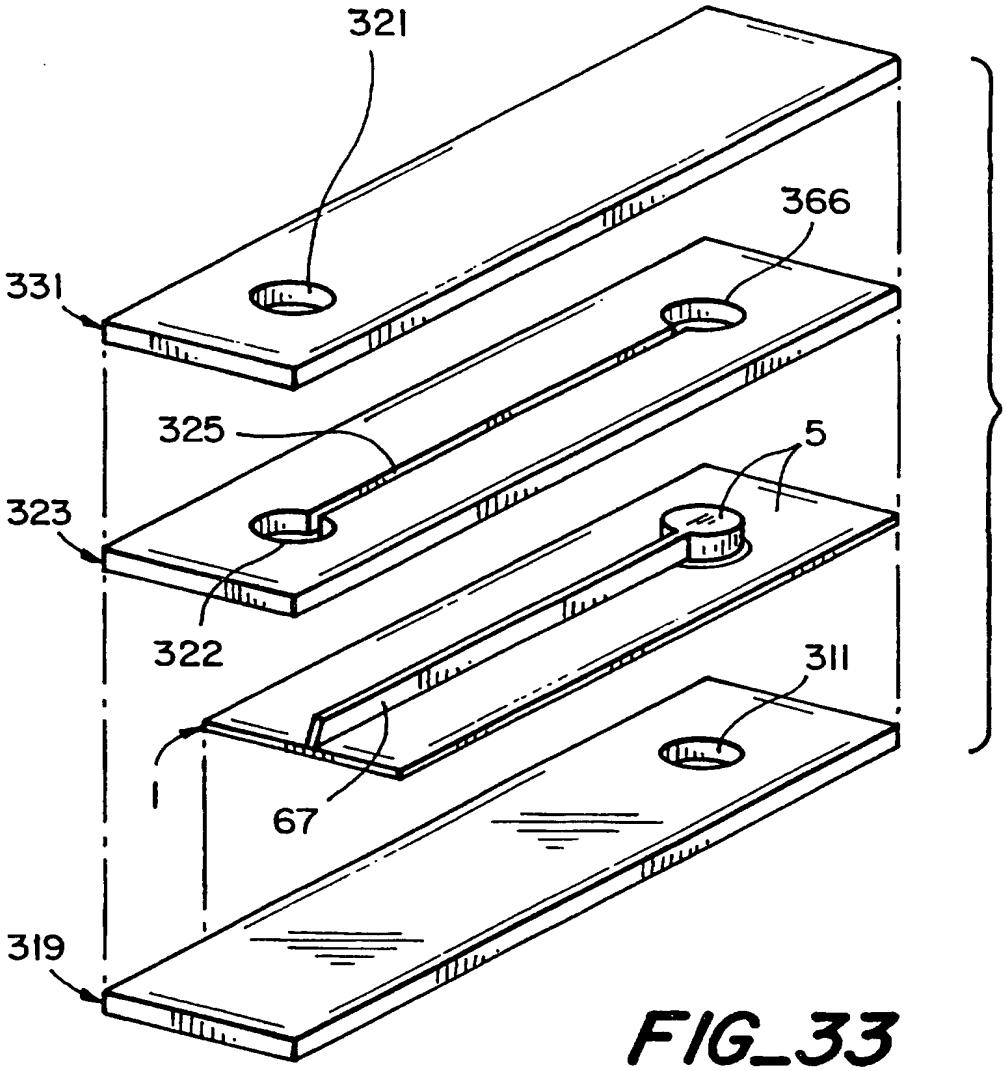


FIG_33

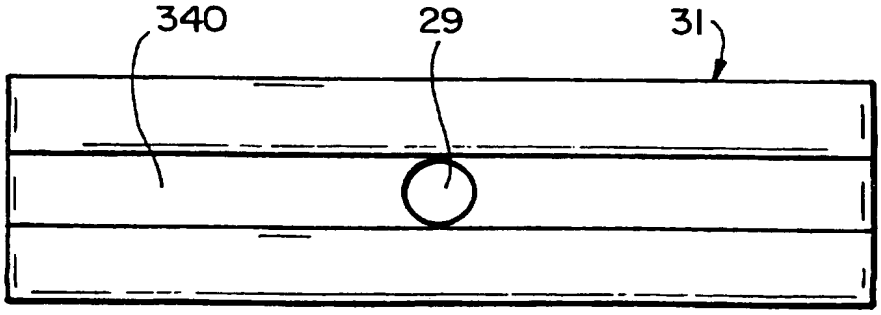


FIG_36

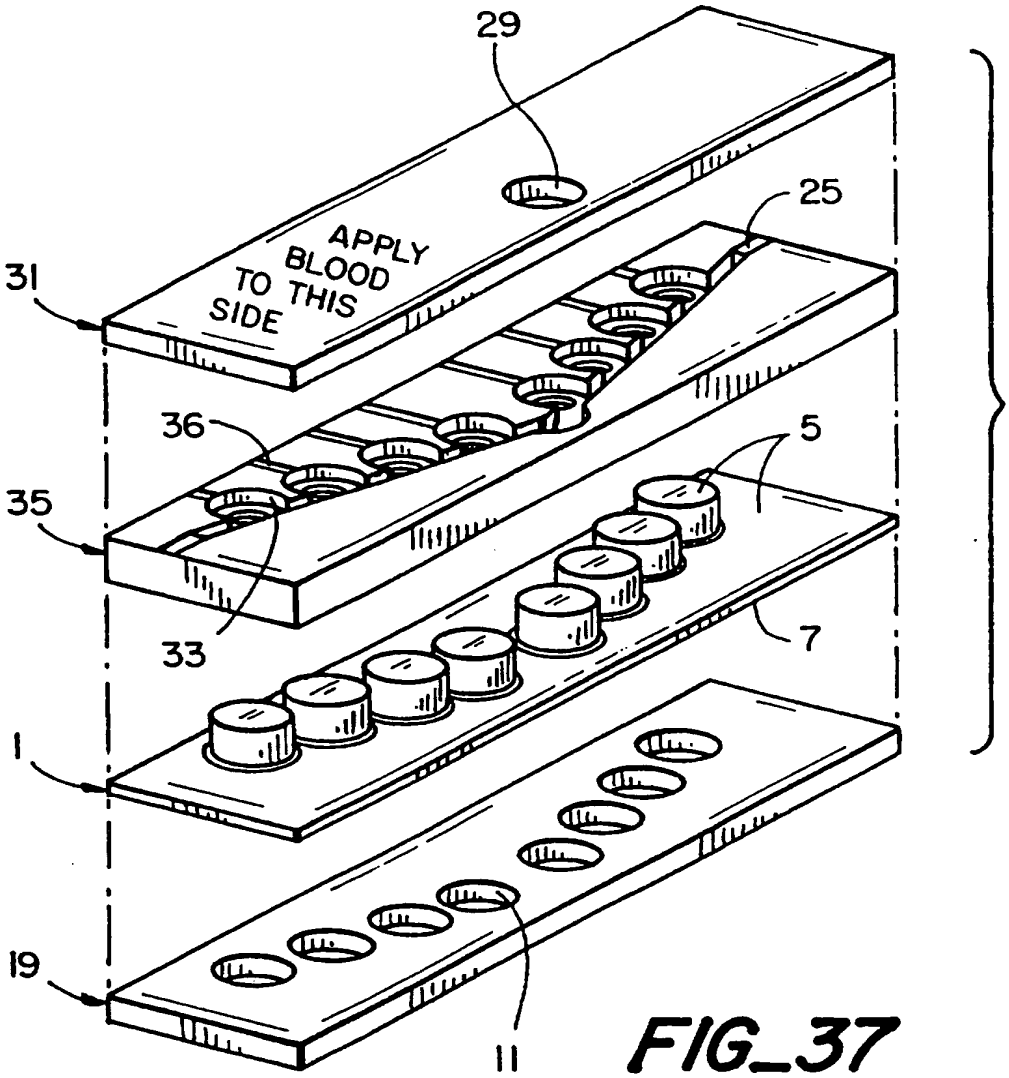


FIG_37

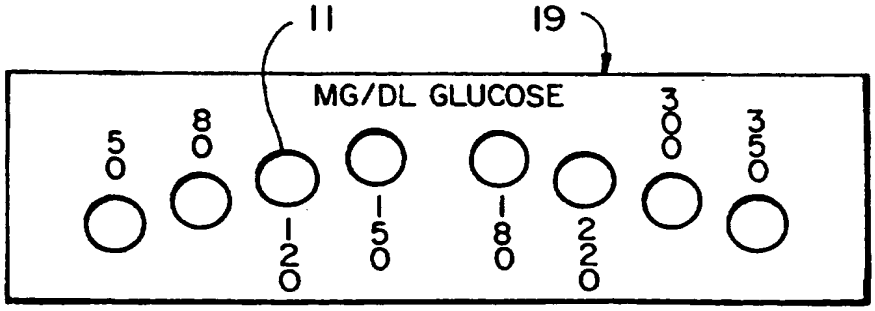


FIG_38

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/05689

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12Q 1/00; G01N 33/48, 33/487, 33/49
US CL :422/50, 61, 68.1; 435/4, 810, 970, 973, 975

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/50, 61, 68.1; 435/4, 810, 970, 973, 975

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, HCAPLUS, WPIDS
search terms: blood test?, fluid test?, device, test strip, analyte, laminat?, matrix

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 3,723,064 A (LIOTTA) 27 March 1973, see entire document.	1-16 and 26-39
A	US 4,678,757 A (RAPKIN et al.) 07 July 1987.	1-39
Y	US 5,215,886 A (PATEL et al.) 01 June 1993, see entire document.	1-39
Y	US 5,330,715 A (BLAKE et al.) 19 July 1994, see entire document.	1-39
A	US 5,504,013 A (SENIOR) 02 April 1996.	1-39
Y	WO 92/17768 A1 (ENVIRONMENTAL TEST SYSTEMS, INC.) 15 October 1992, see entire document.	1-39

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 02 JULY 1997	Date of mailing of the international search report 31 JUL 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer HOWARD C. LEE Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*